

***Escherichia coli* K1 modulates peroxisome proliferator-activated receptor-gamma and glucose transporter-1 at the blood-brain barrier in neonatal meningitis**

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ABSTRACT

Escherichia coli K1 (*E. coli* K1) meningitis continues to be a major threat to neonatal health. Previous studies demonstrated that outer membrane protein A (OmpA) of *E. coli* K1 interacts with Ecgp96 in the blood-brain barrier to enter the central nervous system. Here we show that OmpA-Ecgp96 interaction downregulates the peroxisome proliferator-activated receptor-gamma (PPAR- γ) and glucose transporter-1 (GLUT-1) levels in human brain microvascular endothelial cells (HBMECs) causing disruption of barrier integrity and inhibition of glucose uptake. The suppression of PPAR- γ and GLUT-1 by the bacteria in the brain microvessels of newborn mice causes extensive pathophysiology due to IL-6 production. Pre-treatment with partial or selective PPAR- γ agonists ameliorate the pathological outcomes of infection by suppressing IL-6 production in the brain. Thus, inhibition of PPAR- γ and GLUT-1 by *E. coli* K1 is a novel pathogenic mechanism in meningitis, and pharmacological upregulation of PPAR- γ and GLUT-1 levels may provide novel therapeutic avenues.

Bacterial meningitis is a serious condition that affects the central nervous system. Neonatal and childhood meningitis in particular result in long-term neurological sequelae in about 50% of the survivors [1]. Despite the advent of vaccines and effective antibiotic treatment, there is a recent emergence of clonal variants and antibiotic resistance [2]. One of the central yet incompletely understood dogmas of bacterial meningitis is the reduced glucose levels in the cerebrospinal fluid (CSF) of patients. The CSF glucose levels are typically correlated to serum glucose levels since glucose can be transported across the blood-brain barrier (BBB). A recent clinical study with 3805 normal volunteers with a median age of 2.2 months showed that increased serum glucose levels correlated with increased CSF glucose levels. Therefore, the BBB is the focal point for glucose transport from the blood to the CSF. During bacterial meningitis, the requirement of glucose as a fuel by infiltrating immune cells in response to infection is considered a reason for the reduced glucose levels [3, 4]. However, the possibility of whether bacterial pathogens that cause meningitis can manipulate glucose concentrations in the brain has not been explored before.

Escherichia coli K1 (*E. coli* K1) is the second leading cause of neonatal meningitis, and it uses distinct cellular mechanisms to cross the BBB for disease progression [5]. There is a re-emergence of this pathogen with atypical pathogenic mechanisms [6, 7]. Therefore, it is imperative that our understanding of this pathogen is abreast with its ever-changing virulence strategies. We unequivocally established that the outer membrane protein A (OmpA) of *E. coli* K1 binds endothelial cell glycoprotein 96 (Ecgp96) in both human brain microvascular endothelial cells (HBMECs), an in vitro model of the BBB, and in a newborn mouse model of

meningitis. This interaction is critical for *E. coli* K1 to cross the BBB and establish infection [8-10]. Ecgp96 belongs to the glucose-regulated protein 94 (Grp94) family of heat shock proteins, and therefore its expression is typically induced by lack of glucose or glucose starvation [11]. Our studies also demonstrated the existence of a feedback loop between Ecgp96/TLR2 (Toll-like Receptor-2)/AT1R (Angiotensin II Receptor I) protein complex in the membranes of HBMECs and intracellular nitric oxide (NO) during the invasion process [12-14].

The brain is a glucose-dependent organ since fatty acids cannot cross the BBB [15]. The peroxisome proliferator-activating receptor (PPAR) superfamily of nuclear receptors plays a vital role in cellular glucose uptake by promoting translocation of glucose transporter-1 (GLUT-1) to the membrane and facilitating glucose uptake into the brain from blood vessels [16-18]. Modulation of PPAR- γ and GLUT-1 expression is critical in neurodegenerative disorders [19-24]. Interestingly, the AT1R antagonist Telmisartan (TS), which effectively blocked *E. coli* K1 invasion in vitro and in vivo, also acts as a partial agonist for PPAR- γ activation [14, 25]. Therefore, we speculated that the initial OmpA binding to Ecgp96 triggers a glucose-deficient environment which induces more Ecgp96 expression for bacterial binding and invasion. In this study, we sought to examine the roles of PPAR- γ and GLUT-1 in *E. coli* K1 invasion of HBMECs and the onset of meningitis in newborn mice.

METHODS

Strains and reagents. The prototype *E. coli* K1 strain (O18:K1:H7) and its *ompA* deletion mutant were described previously [26]. The list of antibodies and chemicals used in this study are described in detail in the supplementary information.

Cell culture and infection assays. HBMEC cultures and infection protocols were performed as before [27]. HBMECs were pre-treated with the respective compounds for 1 h before infection. For protein overexpression, HBMECs were transfected with the respective plasmids and invasion assays were performed 24 h post-transfection.

Animal studies, immunostaining, and cytokine ELISA. Animal experiments were performed as described previously [26]. Protocols were approved by the Institutional Animal Care and Use Committee of Children's Hospital of Los Angeles (Protocol #59-14). For treatment studies, the pups were treated with Telmisartan (TS) or Rosiglitazone (RG) 6 h before infection, during infection, and 18 h post-infection. Details are provided in the supplementary information. Cytokine ELISA was performed as per the manufacturer's protocol. Hematoxylin and Eosin (H&E), immunofluorescence, and brain microvessel staining were executed as reported before [14, 24, 26].

Other methods. Western blotting using HBMEC lysates, flow cytometry and measurement of trans-endothelial electrical resistance (TEER) were performed as described earlier [14]. Glucose measurement assays were performed as described earlier [28].

RESULTS

***E. coli* K1 suppresses PPAR- γ and GLUT-1 during the invasion of HBMECs.** To examine if PPAR- γ and GLUT-1 play a role in *E. coli* K1 entry of HBMECs, invasion assays were performed in the presence of TS (partial PPAR- γ agonist/anti-hypertensive drug), RG (selective

PPAR- γ agonist/anti-diabetic drug), GW1929 (selective PPAR- γ agonist), and GW9662 (PPAR- γ antagonist). The IC₅₀ concentrations were determined using dose-dependent studies prior to these experiments (data not shown). Results revealed that pre-treatment of HBMECs with TS, RG, and GW1929 blocked *E. coli* K1 invasion while pre-treatment with PPAR- γ antagonist GW9662 did not have any effect on invasion (Fig. 1A). Western blot analysis of *E. coli* K1-infected HBMEC lysates showed that PPAR- γ expression was suppressed until 30 min, and recovered by 90 min post-infection (Fig. 1B). The expression of GLUT-1 was also significantly suppressed in *E. coli* K1 infected cells as shown by immunoblotting of the infected HBMEC lysates. As previously demonstrated, the expression of Ecgp96, the receptor for *E. coli* K1 OmpA, increased with infection. The expression of AT1R, previously established to be important in the invasion process, did not change with infection. This observation was also consistent with our previous findings that total AT1R levels in HBMECs do not change with *E. coli* K1 invasion, but AT1R association with Ecgp96 increases during the invasion process [14]. No such changes were observed in HBMECs infected with OmpA- *E. coli* K1.

TS or RG pre-treatment did not significantly alter Ecgp96 or AT1R expression after *E. coli* K1 infection of HBMEC compared to *E. coli* K1 alone infected cells. In contrast, pre-treatment with TS or RG inhibited *E. coli* K1-mediated suppression of PPAR- γ and GLUT-1, suggesting that suppression of these two molecules plays a role in the invasion of *E. coli* K1 in HBMECs. To rule out the possible involvement of other PPAR isoforms, we performed invasion assays on HBMECs pre-treated with PPAR- α agonist CP 775146 and PPAR- β agonist L-165,041. As shown in Figure 1C, both the agonists had no effect on the invasion process. Furthermore, to determine if PPAR- α and PPAR- β expression is affected in *E. coli* K1 infected HBMECs,

Western blots were performed using total cell lysates. Both the isoforms were expressed in detectable amounts in the lysates, but the infection process did not alter their levels (Fig. 1D). Additionally, the lysates were also examined for the expression of GLUT-3 and GLUT-4 since both these transporters have been identified at basal levels in the BBB of both humans and rodents [15, 29]. Consistent with this observation, basal levels of GLUT-3 and GLUT-4 were detected in HBMEC lysates, but their levels remained fairly unchanged during the invasion. These results confirm that *E. coli* K1 invasion of HBMECs suppresses the expression of PPAR- γ and GLUT-1. The effect of GLUT-1 agonists on *E. coli* K1 invasion could not be tested due to lack of commercially available compounds that exclusively upregulate GLUT-1. To circumvent this issue, we performed invasion studies in HBMECs overexpressing PPAR- γ and GLUT-1.

Overexpression of recombinant PPAR- γ or GLUT-1 blocks *E. coli* K1 invasion of HBMECs. To confirm the role of PPAR- γ and GLUT-1 in *E. coli* K1 entry of HBMECs, plasmids overexpressing full-length PPAR- γ (FL-PPAR- γ) or GLUT-1 (FL-GLUT-1) were transiently transfected in HBMECs and invasion assays were performed as described above. HBMECs overexpressing full-length GLUT-4 (FL-GLUT-4) were used as a control. Since GLUT-4 mediated glucose uptake is strictly insulin-dependent, it was expected to play no role in glucose uptake in infected HBMECs [15]. *E. coli* K1 invasion was reduced by >50% in HBMECs overexpressing FL-PPAR- γ or FL-GLUT-1, while its invasion of HBMECs overexpressing FL-GLUT-4 was comparable with untransfected or transfection reagent alone-treated HBMECs (Fig. 2A). The overexpression of these proteins in transfected HBMECs were also comparable as verified by flow cytometry (Fig. 2B). Western blot analyses of lysates from transfected HBMECs infected with *E. coli* K1 showed that the expressions of Ecgp96, AT1R,

PPAR- γ , and GLUT-1 were similar to those observed with RG pre-treated/*E. coli* K1-infected HBMECs (Fig. 2C). These observations confirm that suppression of PPAR- γ and GLUT-1 levels either by RG pre-treatment or with plasmid-mediated overexpression blocks *E. coli* K1 invasion.

***E. coli* K1 suppresses glucose uptake and induces tight junction disruption in HBMECs**

during the invasion. To demonstrate the direct role of glucose uptake in HBMECs in preventing *E. coli* K1 invasion, a colorimetric assay to quantify 2-deoxyglucose (2-DG) uptake was performed. [28]. *E. coli* K1 suppressed glucose uptake in HBMECs in comparison to uninfected control HBMECs. Treatment with TS or RG before infection with *E. coli* K1 rescued the glucose uptake levels to those seen in uninfected controls (Fig. 3A). In contrast, overexpression of FL-PPAR- γ or FL-GLUT-1 enhanced glucose levels despite infection with *E. coli* K1 when compared to those levels in HBMECs infected with the bacteria alone (Fig. 3B). These observations indicate a direct correlation between PPAR- γ , GLUT-1, and glucose uptake, and confirm our hypothesis that *E. coli* K1 suppresses glucose uptake mechanisms mediated by PPAR- γ and GLUT-1 in HBMECs during infection.

The expression of PPAR- γ and GLUT-1 is vital for the barrier integrity of the BBB [24, 30]. We previously reported that disruption of tight junction (TJ) and adherens junction (AJ) integrity plays a key role in *E. coli* K1 translocation across the HBMEC monolayer [27, 31]. Moreover, pre-treatment of HBMECs with TS completely inhibited *E. coli* K1-mediated barrier disruption [14]. To determine whether the selective PPAR- γ agonist RG or overexpression of PPAR- γ and GLUT-1 plasmids also prevented junction disruption, trans-endothelial electrical resistance

(TEER) was measured in *E. coli* K1-infected HBMECs. Similar to TS, RG pre-treatment or plasmid overexpression also inhibited the ability of *E. coli* K1 to disrupt barrier integrity (Fig. 3C-D). Therefore, *E. coli* K1 signals for barrier disruption in HBMECs through the Ecgp96/AT1R/TLR2 complex, and this process involves suppression of PPAR- γ and/or GLUT-1.

Specific activation of PPAR- γ prevents the onset of meningitis in newborn mice. Our results show that PPAR- γ and GLUT-1 may play a role in modulating glucose uptake in HBMECs, an in vitro model of BBB during *E. coli* K1 invasion. To establish the exclusive role of PPAR- γ in a newborn mouse model of *E. coli* K1 meningitis, infection experiments with *E. coli* K1 were performed in 2-day-old mouse pups in the presence or absence of RG pre-treatment as described in methods. TS-S (TS-sulfate, a soluble ester of TS) pre-treatment was used as a control based on our earlier observations that TS-S prevented the onset of meningitis in vivo [14]. Newborn mice infected with *E. coli* K1 were positive for bacteria in the blood, brain, and CSF by 24 h post-infection. In contrast, pups pre-treated with TS or RG before infection were resistant to meningitis and survived (Fig. 4A-C). Hematoxylin and Eosin (H&E) staining of the brain sections revealed that cortex, meninges, and hippocampus regions of the infected brains were severely affected while brains from uninfected and compound pre-treated pups showed no such damage (Fig. 4D).

***E. coli* K1 alters pro-inflammatory cytokine profiles in the serum and brain of newborn pups.** *E. coli* K1 infection causes brain damage similar to the human disease in infected mouse pups as evidenced by pathological damage and neutrophil infiltration [26, 32, 33]. H&E staining

revealed severe brain pathology in infected pups due to the excessive neutrophil influx caused by pro-inflammatory cytokines. To identify the pattern of cytokine expression concurrent with disease progression, multiplex cytokine ELISA was performed to check for IL-6, IL-1 β , TNF α , IL-12p70, IFN γ , IL-4, IL-5, and IL-10 expression in serum samples and brain homogenates. Results showed that *E. coli* K1 induces pro-inflammatory IL-6, IL-1 β , and TNF α levels in serum samples (Fig. 5A-B). No significant changes in IL-12p70, IFN γ , IL-4, IL-5, and IL-10 expression were detected. However, only IL-6 was significantly upregulated in the brains of infected pups. Pre-treatment with TS or RG inhibited the upregulation of IL-6, IL-1 β , and TNF α levels in the serum. These results show that *E. coli* K1 induces a typical “cytokine storm” which primarily involves IL-6 to potentially cause inflammation-mediated damage in the brains of infected mice. Interestingly, TS or RG failed to induce a compensatory mechanism of anti-inflammatory cytokine activation (IL-4, IL-5, and IL-10). Rather, the compounds directly suppressed *E. coli* K1-mediated IL-6 upregulation, which may play a role in the eventual clearance of the bacteria from the systemic circulation, thus preventing the onset of meningitis.

***E. coli* K1 infection causes reciprocal regulation of Ecgp96/AT1R and PPAR- γ /GLUT-1**

levels in the brains of mouse pups. Our in vitro data show that *E. coli* K1 suppresses the expression of PPAR- γ and GLUT-1 in HBMECs. Therefore, we examined if *E. coli* K1 infection also causes suppression of these molecules at the BBB in vivo. We previously demonstrated that the *E. coli* K1 infection affects cortex, meninges, and hippocampus regions of the brain [9, 26]. Moreover, we showed that Ecgp96 levels are upregulated in these regions of the brain during *E. coli* K1 infection [9]. To determine whether elevated Ecgp96 levels lead to a concomitant upregulation of AT1R and suppression of PPAR- γ and GLUT-1 levels, we

determined the expression of Ecgp96, AT1R, PPAR- γ , and GLUT-1 in the brain sections by immunofluorescence. Consistent with previous observations, Ecgp96 levels were elevated in the meninges and hippocampus of infected pups, as were AT1R levels (Fig. 6A-B) [9]. However, PPAR- γ and GLUT-1 levels were downregulated in the *E. coli* K1-infected brains. In contrast, Ecgp96 and AT1R levels were basal, while PPAR- γ and GLUT-1 levels were higher in TS-S and RG + *E. coli* K1 pups than in uninfected pups. Compound alone-treated pups (no infection) also showed elevated levels of PPAR- γ and GLUT-1 (data not shown), implying that upregulation of their levels using chemical agonists prevented bacterial translocation across the BBB. To examine the expression of these markers at the BBB, brain microvessels were stained with tomato lectin in the brain sections. Consistent with the patterns above, the expression of Ecgp96 and AT1R was upregulated in the microvessels of infected tissue while PPAR- γ and GLUT-1 levels were downregulated (Fig. 6C-D). An inverse phenomenon was observed in the brain microvessels of TS-S and RG + *E. coli* K1 pups compared to *E. coli* K1-infected mouse brains. The localization of Ecgp96, AT1R, and GLUT-1 to the membrane of the microvessels and cytoplasmic expression of PPAR- γ are clearly observed. These results suggest that *E. coli* K1 invasion of brain microvessels downregulates PPAR- γ and GLUT-1 expression to infect deeper tissue.

DISCUSSION

Most *E. coli* strains that cause meningitis contain K1 capsular polysaccharide, which is shown to be necessary for survival in the host [34]. We demonstrated that OmpA is also important for the survival of *E. coli* K1 strains [10]. Mutation in extracellular loops 1 and 2 of OmpA prevented meningitis in newborn mice. These two loops have slight differences in their sequences

compared to non-pathogenic *E. coli*. Moreover, they interact with the N-glycans of Ecgp96 to enter HBMECs [9]. Since Ecgp96 upregulation has been correlated with glucose starvation, we analyzed whether alterations of glucose uptake mechanisms mediated by the OmpA-Ecgp96 interaction play a role in *E. coli* K1 meningitis.

The promotion of glucose uptake by PPAR- γ and GLUT-1 has been a well-established paradigm in the maintenance of brain health. [15, 35-37]. However, the role of PPAR- γ and/or GLUT-1 in meningitis has not been explored previously. The data presented here using in vitro and in vivo models clearly indicate a critical role played by these markers in the development of neonatal meningitis by *E. coli* K1. We also propose an inverse correlation between Ecgp96 and PPAR- γ wherein the binding of *E. coli* K1 OmpA to basal levels of Ecgp96 causes suppression of PPAR- γ . This leads to inhibition of glucose uptake due to the downregulation of GLUT-1 expression. This glucose-deficient environment leads to a stress response in the BBB, thereby further enhancing the expression of Ecgp96 for more bacteria to bind and invade.

Pathogens including *Brucella*, *Listeria*, and enteroaggregative *E. coli* have been recently shown to upregulate PPAR- γ [28, 38, 39]. On the contrary, we demonstrated here that *E. coli* K1 suppresses PPAR- γ and GLUT-1 levels in the endothelial cells forming the BBB, and upregulation of PPAR- γ (using partial and selective PPAR- γ agonists) prevented *E. coli* K1 translocation of the BBB to establish infection. The role of PPAR- γ and GLUT-1 in the maintenance of tight junction (TJ) and adherens junction (AJ) integrity is well recognized [40, 41]. Furthermore, we previously showed that *E. coli* K1 disrupts AJ during the invasion of the BBB [27, 31]. Our current results demonstrated that pharmacological augmentation of PPAR- γ

levels using TS or RG prevents AJ disruption, and therefore manipulation of PPAR- γ levels by *E. coli* K1 may be a critical step in the invasion process. We showed that TS pre-treatment inhibited *E. coli* K1-mediated AJ disruption [14]. Our current data further confirms that TS exerts its activity as an inhibitor of bacterial invasion by both AT1R antagonism and PPAR- γ upregulation. Furthermore, this dual activity of TS was helpful in the establishment of a previously unknown signaling link between *E. coli* K1 OmpA and its receptor Ecgp96 (along with AT1R and TLR2), PPAR- γ , and endothelial barrier integrity.

Disruption of BBB integrity causes an influx of inflammatory mediators that cause extensive tissue damage [42]. *E. coli* K1 causes brain damage by inducing neuronal apoptosis and neutrophil infiltration in the brains of infected mouse pups [26]. However, the specific inflammation patterns underlying the observed pathology in either humans or mice are not well studied. Our results clearly show a prominent role of the pro-inflammatory cytokine IL-6 as a mediator of brain damage. Interestingly, IL-6 levels were also elevated in meningitis caused by Group B Streptococcus (GBS) and *Haemophilus influenzae* type B (Hib) [43, 44]. Moreover, increased IL-6 production by astrocytes and microglia during brain injury or meningitis leads to increased influx of leukocytes into the brain [45, 46]. Therefore, neutrophil infiltration during *E. coli* K1 meningitis may be a result of IL-6 production. IL-6 upregulation in the brain corresponding to the lack of PPAR- γ activity was recently shown in Alzheimer's disease [47]. In agreement, preservation of PPAR- γ activity by pre-treatment with TS or RG effectively inhibited *E. coli* K1-mediated IL-6 production, promoted bacterial clearance, and prevented inflammation-related pathology. Another interesting observation here is that though *E. coli* K1-mediated suppression of PPAR- γ and GLUT-1 levels is consistent in vitro and in vivo, *E. coli* K1 does not

induce cellular damage in HBMECs even after 24 h-post infection (unpublished observation). Therefore, the extensive tissue damage seen in vivo maybe the result of IL-6 induction due to lack of PPAR- γ activity during infection. However, further studies using IL-6 knockout mice are needed to conclusively demonstrate the role of IL-6 in *E. coli* K1-induced brain damage.

The current study unravels a unique infection strategy employed by *E. coli* K1 in the pathogenesis of neonatal meningitis by manipulating PPAR- γ and GLUT-1 levels. The modulation of these proteins exhibits a causal effect on barrier disruption and inhibition of glucose uptake, thereby providing new avenues for therapeutic approaches. The inhibition of glucose uptake at the BBB could be an additional factor contributing to the low glucose levels in the CSF during *E. coli* K1 meningitis. The clinical diagnosis of meningitis is based on CSF-positive cultures for *E. coli* K1. However, the study involves pre-treatment with PPAR- γ agonists before experimentally inducing meningitis. Therefore, the most credible therapeutic strategies would involve the use of antibiotics in conjunction with TS or RG to override suppression of glucose uptake by *E. coli* K1. Our unpublished observations reveal that supplementing antibiotic treatment with TS results in a quicker resolution of brain inflammation in pups compared to those treated and rescued with antibiotics alone. This short-term antibiotic/TS treatment does not seem to have lasting sequelae on these animals when they eventually reach adulthood. Clinical studies indeed show that short-term treatment regimens (ranging from 3 weeks to 24 weeks) of the anti-hypertensive drug Candesartan (an angiotensin receptor blocker similar to TS) in children with hypertension (ages 1 to 18 years) does not cause any adverse effects [48]. Since brain inflammation during meningitis causes intracranial hypertension, adjunct treatment with TS may also help resolve intracranial hypertension along

with suppression of IL-6 levels leading to better brain recovery [49]. It is unclear if the effects of TS or RG on PPAR- γ in other cell types contribute to the reduction in blood and brain bacterial levels in *E. coli* K1-infected mice. Further studies using brain endothelial cell specific PPAR- γ knockout mice are needed to validate the role of PPAR- γ in the onset of meningitis. Nevertheless, therapeutic intervention with PPAR- γ agonists along with antibiotics may eventually evolve into an effective method of preventing morbidity and neurological sequelae in meningitis due to *E. coli* K1 and perhaps other bacterial pathogens.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

FUNDING STATEMENT

This work was supported by funds from NIAID (AI40567) and NICHD (NS73115) to NVP.

ACKNOWLEDGMENTS

We sincerely thank G. Esteban Fernandez for assistance with confocal imaging.

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Figure 1. *E. coli* K1 suppresses PPAR- γ and GLUT-1 expression during the invasion of HBMECs. (A) Bacterial invasion assays in HBMECs were performed using the following concentrations of TS (39 μ M), RG (50 μ M), GW1929 (50 μ M), and GW9662 (33 nM). Data represent relative invasion compared to *E. coli* K1 in the presence of DMSO taken as 100% and are averages of three independent experiments. Error bars indicate standard deviation. $*p < 0.05$ by Student's *t*-test compared to DMSO. (B) Lysates from HBMECs exposed to bacteria for up to 90 min in the presence or absence of compound pre-treatment for 1 h were probed for expression of Ecgp96, PPAR- γ , GLUT-1, and AT1R. β -actin was used as a loading control; C indicates uninfected control. The blots shown are representative of two independent experiments with similar results. (C) Bacterial invasion assays on HBMECs were performed using the following concentrations of PPAR- α agonist CP 775146 (24.5 nM) or PPAR- β agonist L-165,041 (125 nM). Data represent relative invasion compared to *E. coli* K1 in the presence of DMSO taken as 100% and are averages of three independent experiments. Error bars indicate standard deviation. (D) Expression levels of PPAR- α , PPAR- β , GLUT-3, and GLUT-4 were analyzed from lysates of HBMECs infected with *E. coli* K1 for various time points as indicated. β -actin was used as a loading control; C indicates uninfected control. The blots shown are representative of two independent experiments that exhibited similar results.

Figure 2. Overexpression of recombinant PPAR- γ or GLUT-1 blocks *E. coli* K1 invasion of HBMECs. (A) HBMECs transfected with plasmids expressing full-length (FL)-PPAR- γ , (FL)-GLUT-1, or (FL)-GLUT-4 were subjected to invasion assays with *E. coli* K1. Data represent relative invasion compared to *E. coli* K1 in the presence of Lipofectamine 3000 (Lipo) taken as 100% and are averages of three independent experiments. Error bars indicate standard deviation. $*p < 0.05$ by Student's *t*-test compared to Lipofectamine 3000 (Lipo). (B) Flow cytometry

analysis of untransfected and plasmid-transfected HBMECs was performed using respective antibodies. Data are representative of two independent experiments with similar results. (C) Lysates from untransfected or plasmid-transfected HBMECs exposed to bacteria for various time points as indicated were probed for expression of Ecgp96, PPAR- γ , GLUT-1, and AT1R. β -actin was used as a loading control; C indicates plasmid-transfected/uninfected control. The blots shown are representative of two independent experiments.

Figure 3. *E. coli* K1 suppresses glucose uptake and induces monolayer permeability in HBMECs during the invasion. HBMECs were pre-treated with (A) compounds or (B) transfected with plasmids and infected with *E. coli* K1. Colorimetric assessment of glucose uptake was measured using 2-deoxyglucose as substrate. In these graphs, the same results obtained with uninfected HBMECs (uninfected) and *E. coli* K1-infected HBMECs (untreated) were used in both panels for clarity. Error bars indicate standard deviation. $\#p < 0.05$ by Student's *t*-test compared to uninfected; $*p < 0.05$ by Student's *t*-test compared to Lipofectamine 3000 (Lipo). HBMECs were pre-treated with (C) compounds or (D) transfected with plasmids, and infected with *E. coli* K1 for measurement of trans-endothelial electrical resistance (TEER) at various time points. In these graphs, the same results obtained with uninfected HBMECs (uninfected) and *E. coli* K1-infected HBMECs (*E. coli* K1) were used in both TEER experiments for clarity. Error bars indicate standard deviation. $*p < 0.05$ by Student's *t*-test compared to *E. coli* K1 from 30 to 120 min only.

Figure 4. Activation of PPAR- γ prevents the onset of meningitis in newborn mice. Two-day-old mice were infected with *E. coli* K1 via an intraperitoneal route as described in the Methods. Blood samples (A) and brains (B) from infected mice were collected 24 h post-infection and bacterial load was quantified. Statistical analyses were performed by Student's *t*-

test (p -values indicated); ANOVA verified the significant differences in the means. (C) CSF samples were collected and determined for the presence of bacteria as described in the Methods. Positive CSF cultures were indicative of meningitis. (D) Brain sections were stained for pathological analysis using Hematoxylin & Eosin staining. Arrows indicate normal (uninfected, TS-S + *E. coli* K1, RG + *E. coli* K1) or damaged (*E. coli* K1) meninges and hippocampi. Scale bar = 100 μ m.

Figure 5. *E. coli* K1 modulates cytokine profiles in newborn mice. Serum samples (A) and brain homogenates (B) were used for cytokine analysis using ELISA. Statistical analyses were performed by Student's t -test and ANOVA; $*p < 0.05$ by Student's t -test compared to uninfected (UN); $\#p < 0.05$ by Student's t -test compared to *E. coli* K1-infected samples (E).

Figure 6. *E. coli* K1 promotes reciprocal regulation of Ecgp96/AT1R and PPAR- γ /GLUT-1 levels in the brains of newborn mice. The brain sections were stained with antibodies to Ecgp96, AT1R, PPAR- γ , and GLUT-1 followed by Alexa 647 fluorophore-coupled (red) secondary antibodies. (A) Meninges and (B) hippocampus are shown for each treatment. DAPI (blue) was used to stain the nuclei. Scale bar = 100 μ m. Additionally, the brain sections were stained with tomato lectin (green) to visualize microvessels and for (C) Ecgp96, AT1R, (D) PPAR- γ , and GLUT-1 (red) expression in the meninges. DAPI (blue) was used to stain the nuclei. Scale bar = 20 μ m.

Fig. 1

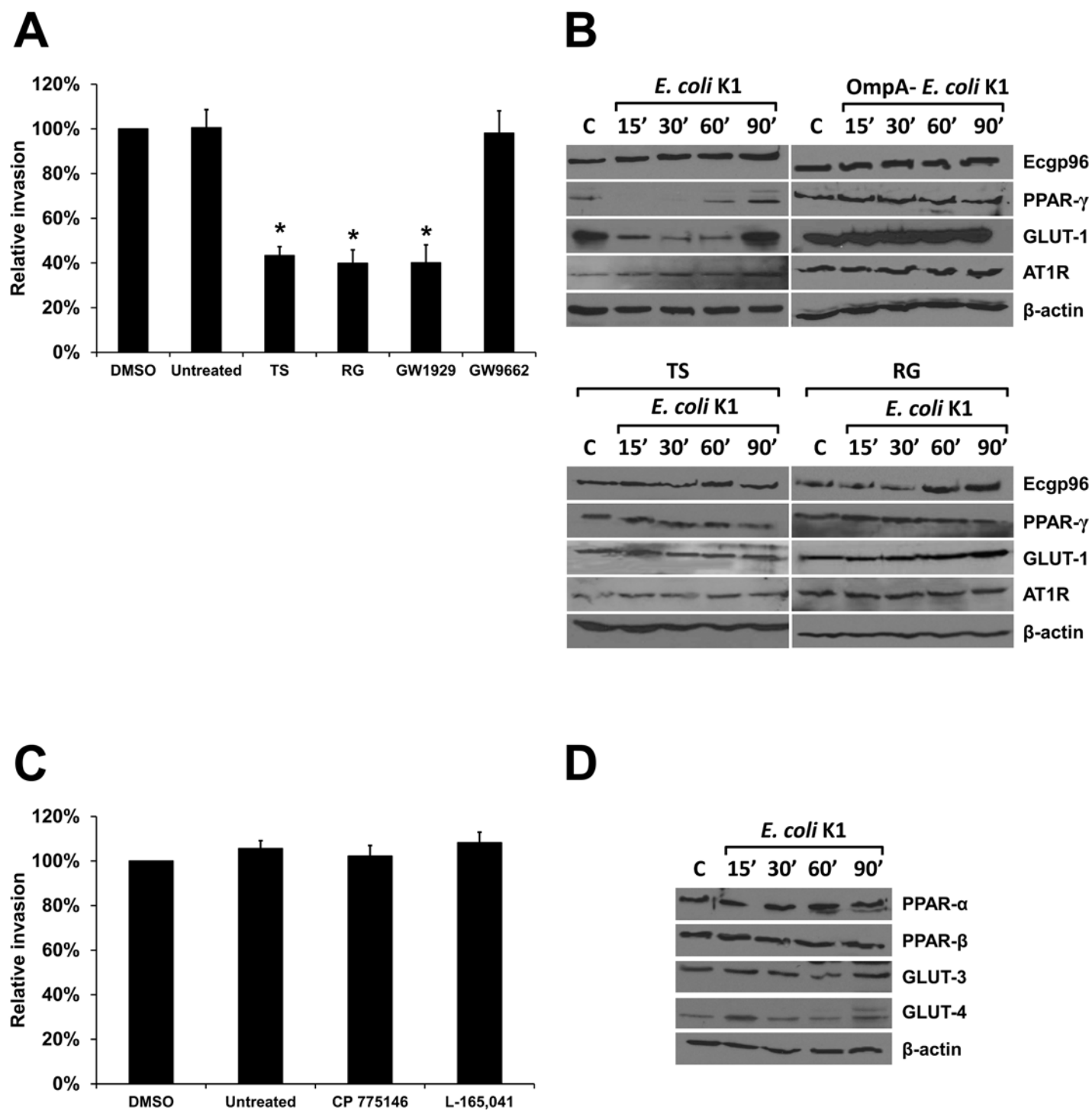


Fig. 2

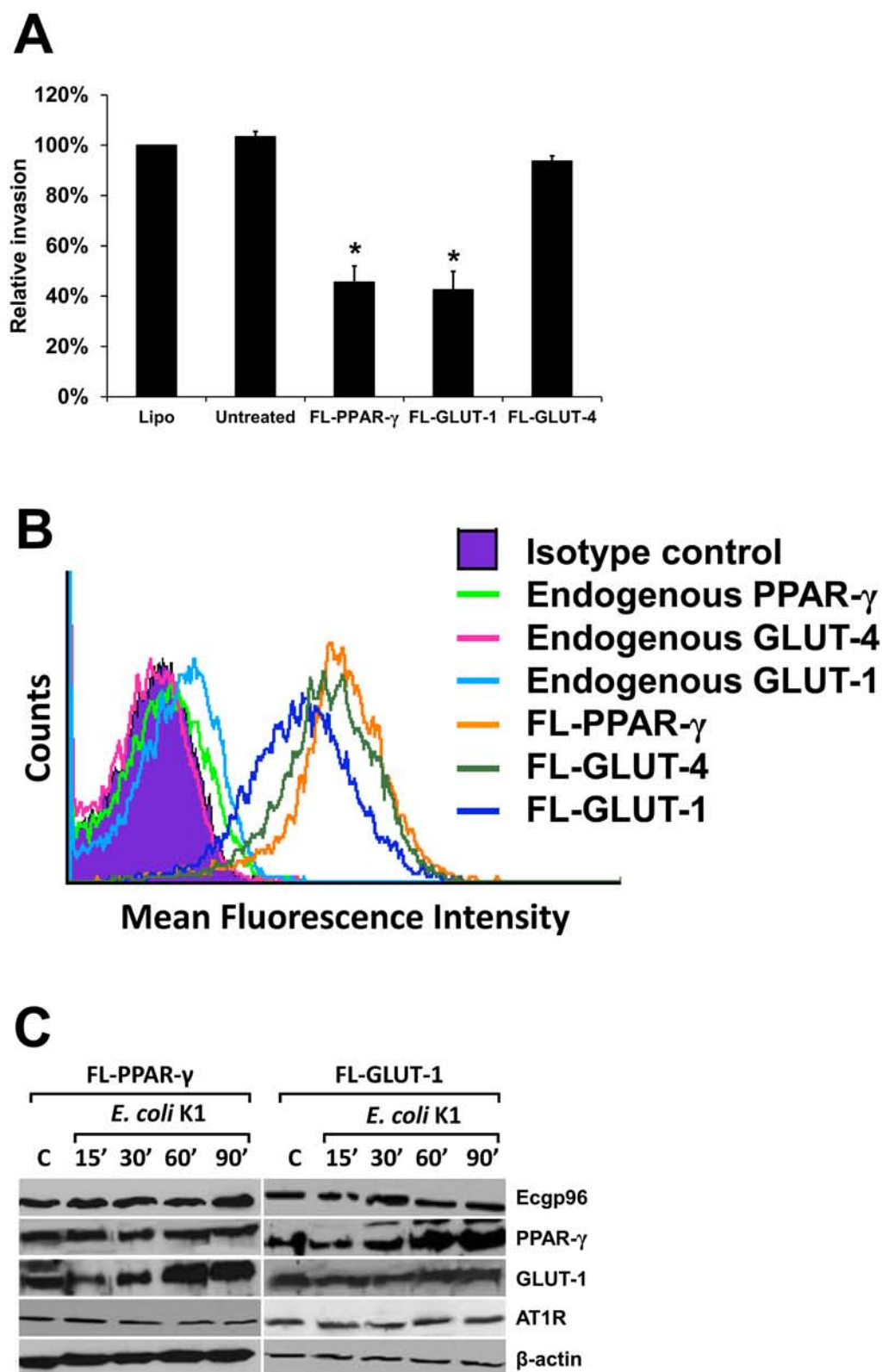


Fig. 3

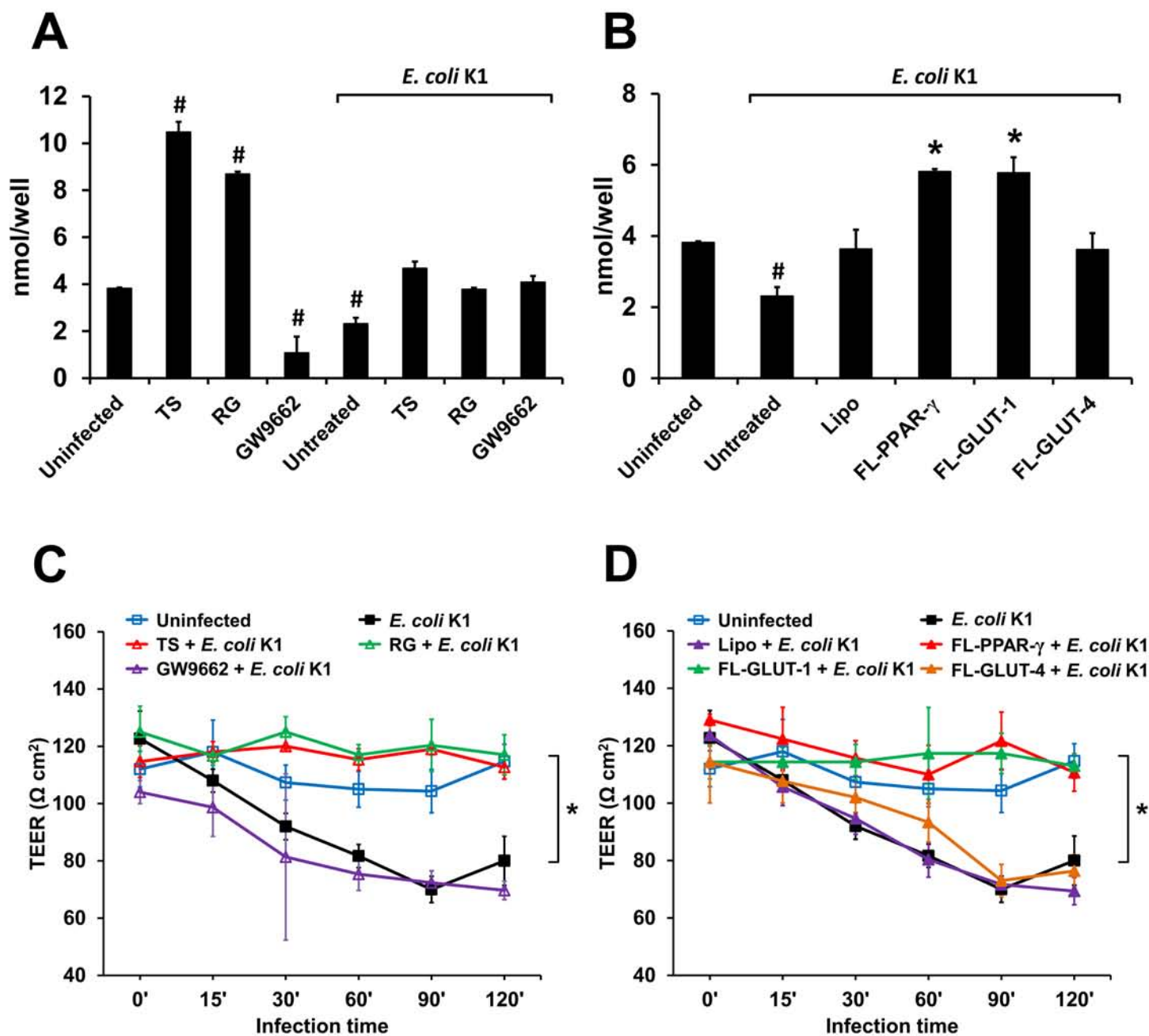
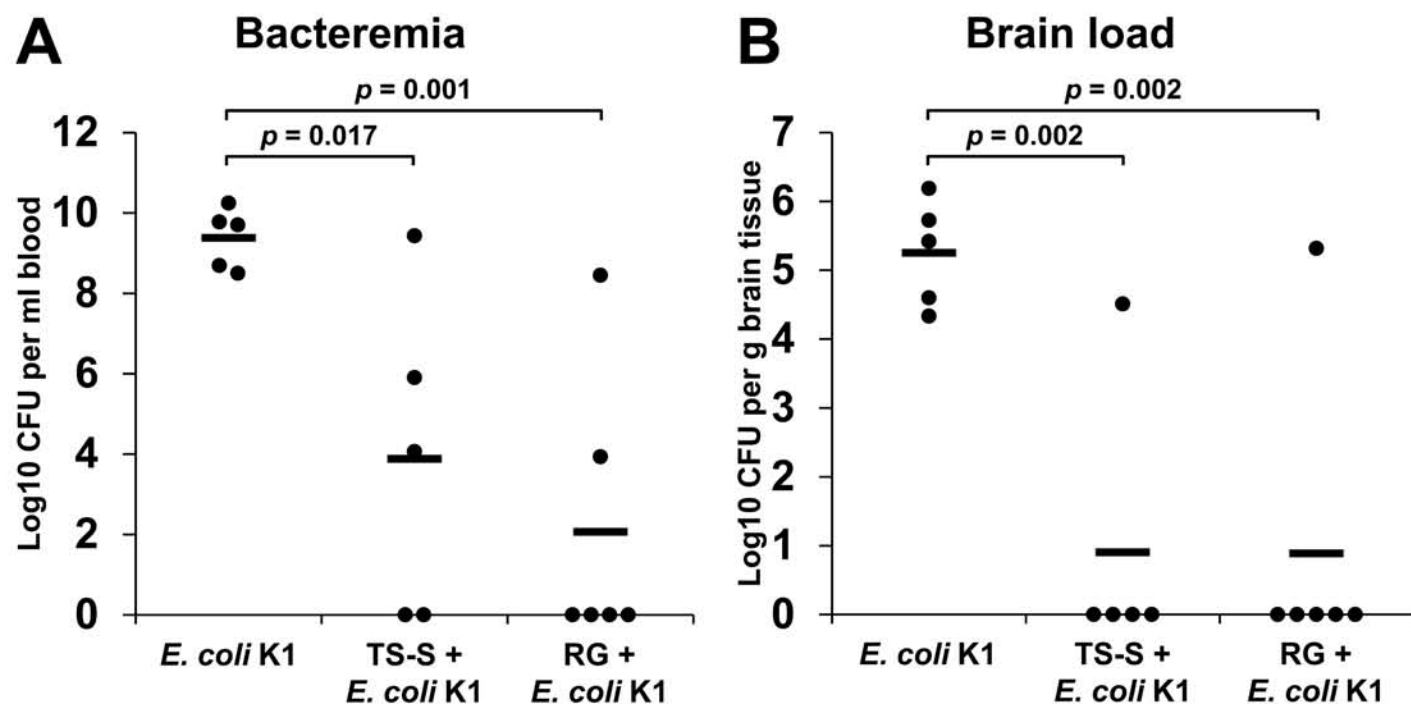


Fig. 4



C

Strain	Mortality rate*	Bacteremia	Meningitis (CSF positive)	Mean blood (log ₁₀ CFU/ml)	Mean brain (log ₁₀ CFU/g)
<i>E. coli</i> K1	60%	100%	100%	9.38	5.25
TS-S + <i>E. coli</i> K1	0%	60%	20%	3.88	0.90
RG + <i>E. coli</i> K1	0%	33%	17%	2.06	0.89

*All animals were monitored for symptoms up to 24 hours post-infection.

D

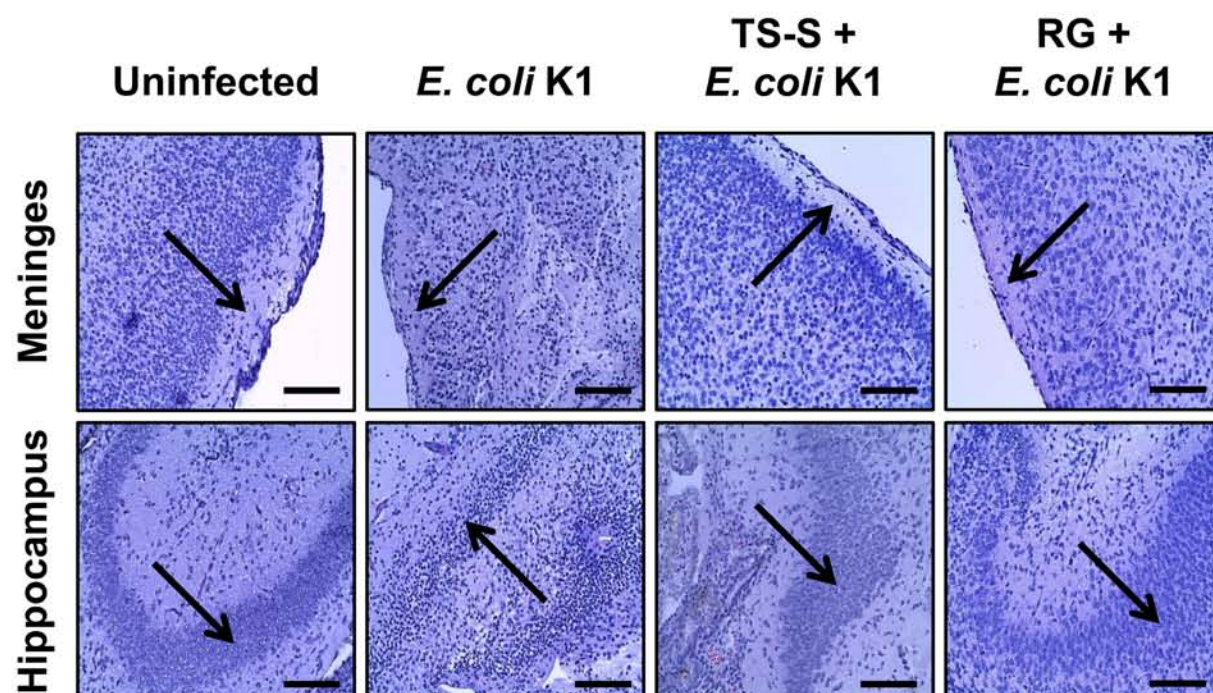
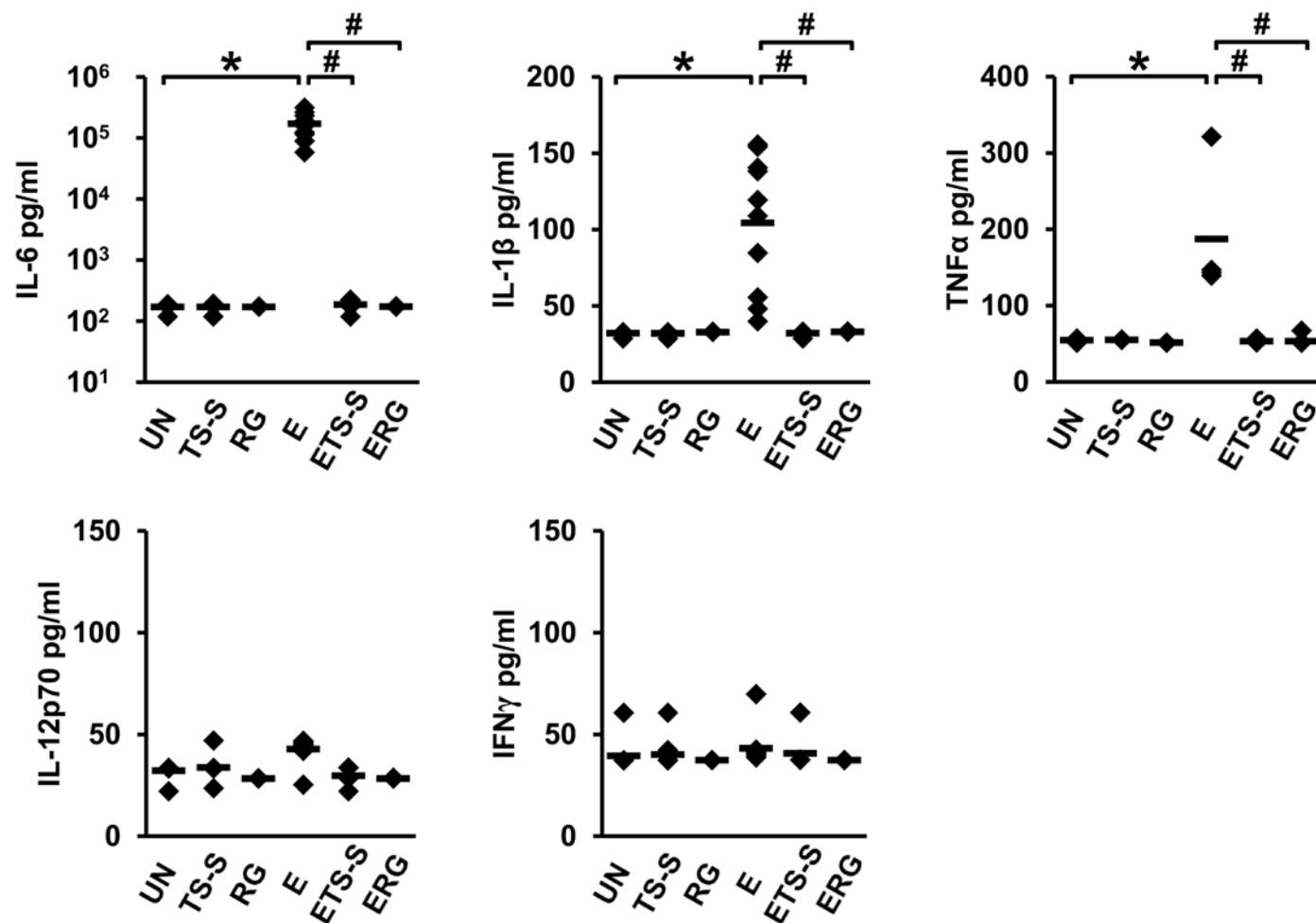


Fig. 5

A

Pro-inflammatory Cytokines



Anti-inflammatory Cytokines

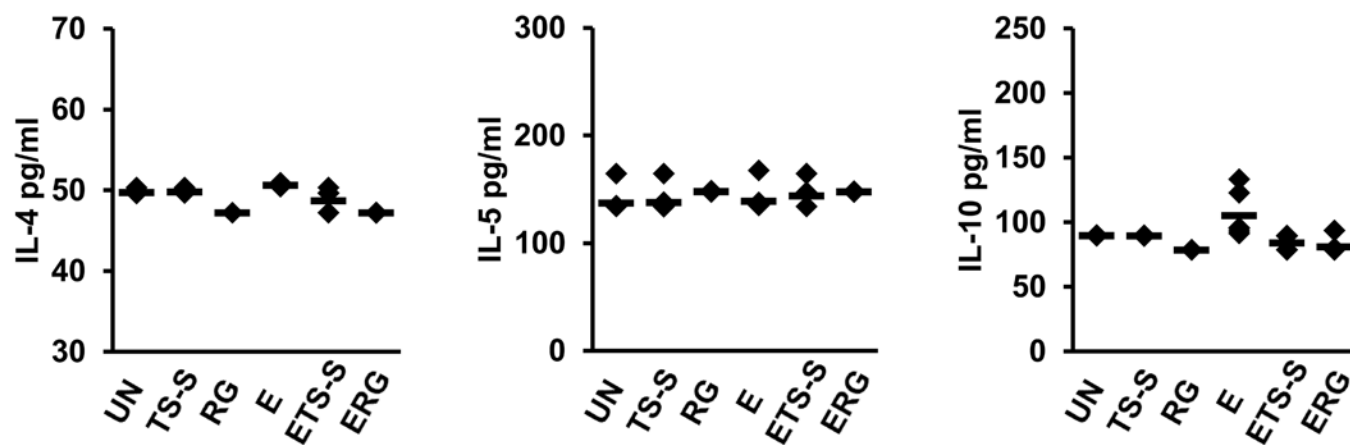
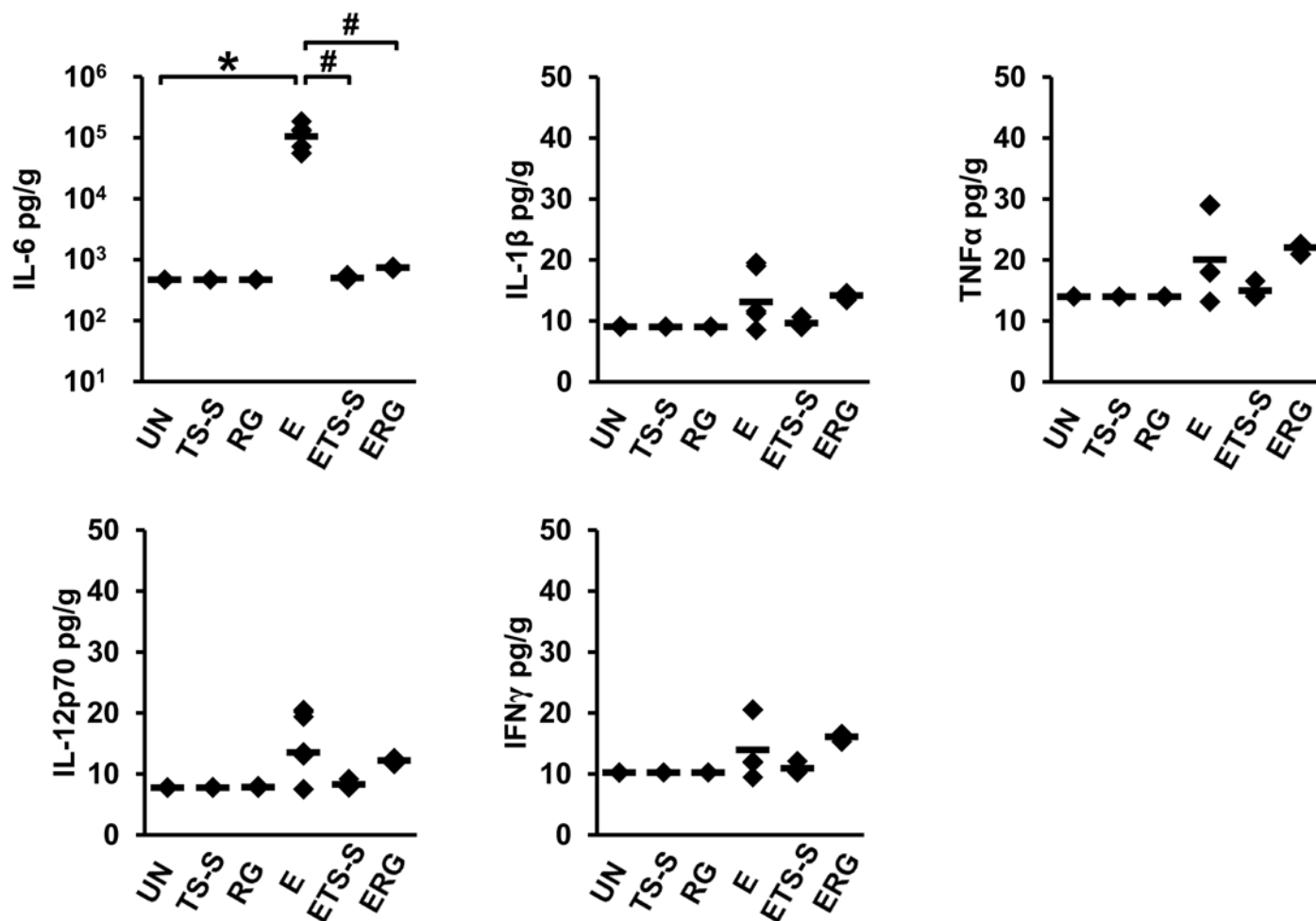


Fig. 5

B

Pro-inflammatory Cytokines



Anti-inflammatory Cytokines

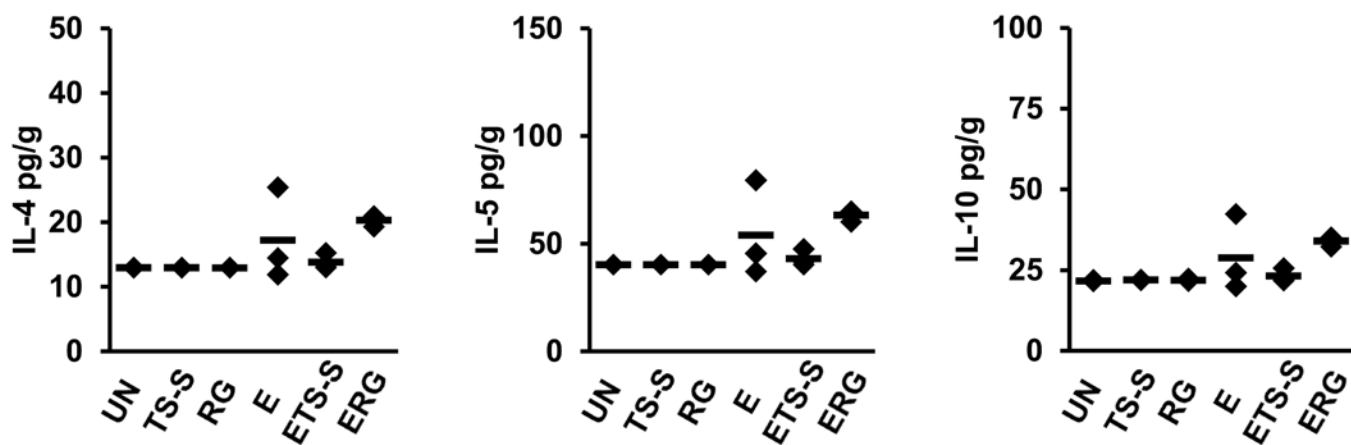


Fig. 6

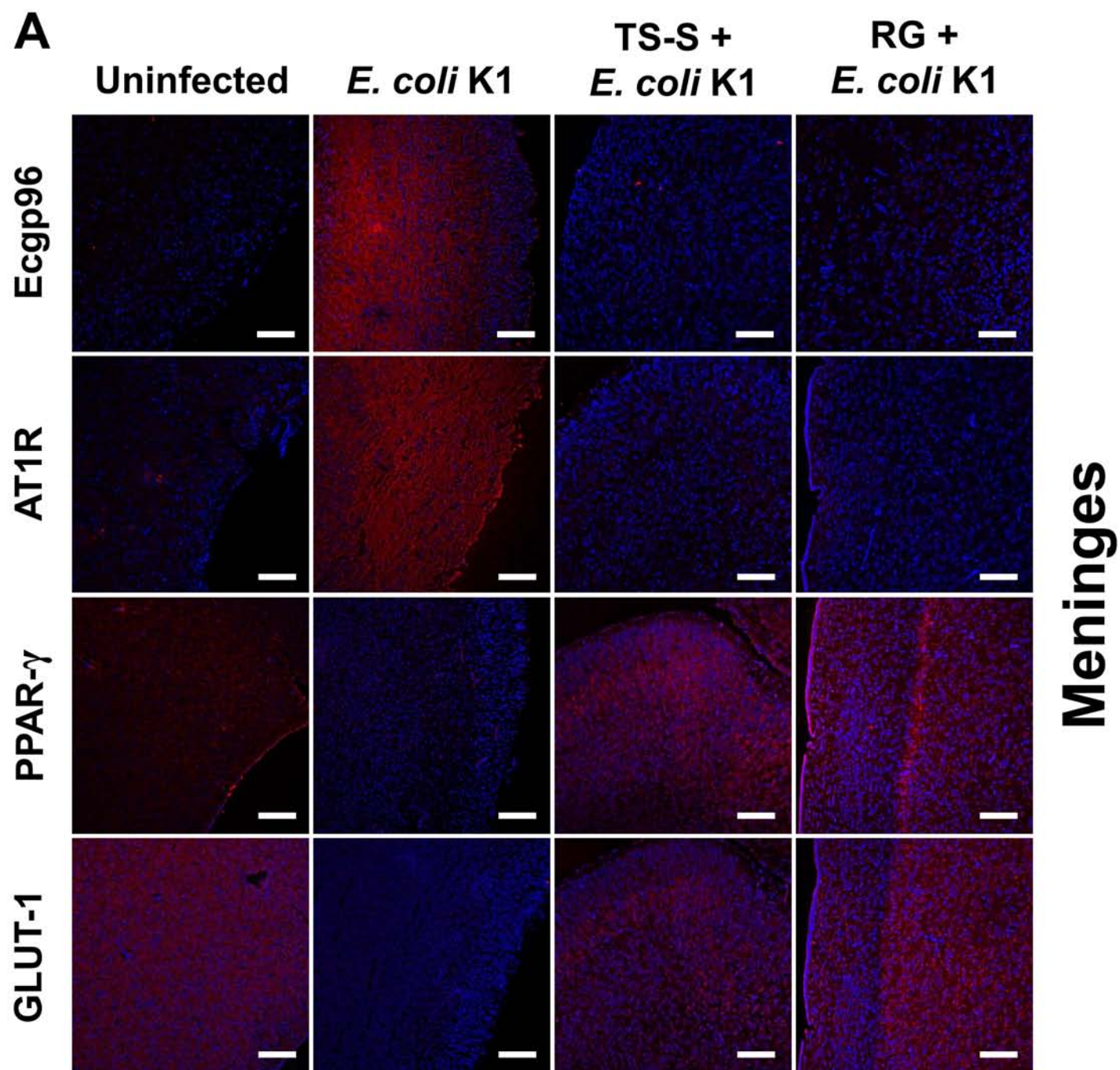


Fig. 6

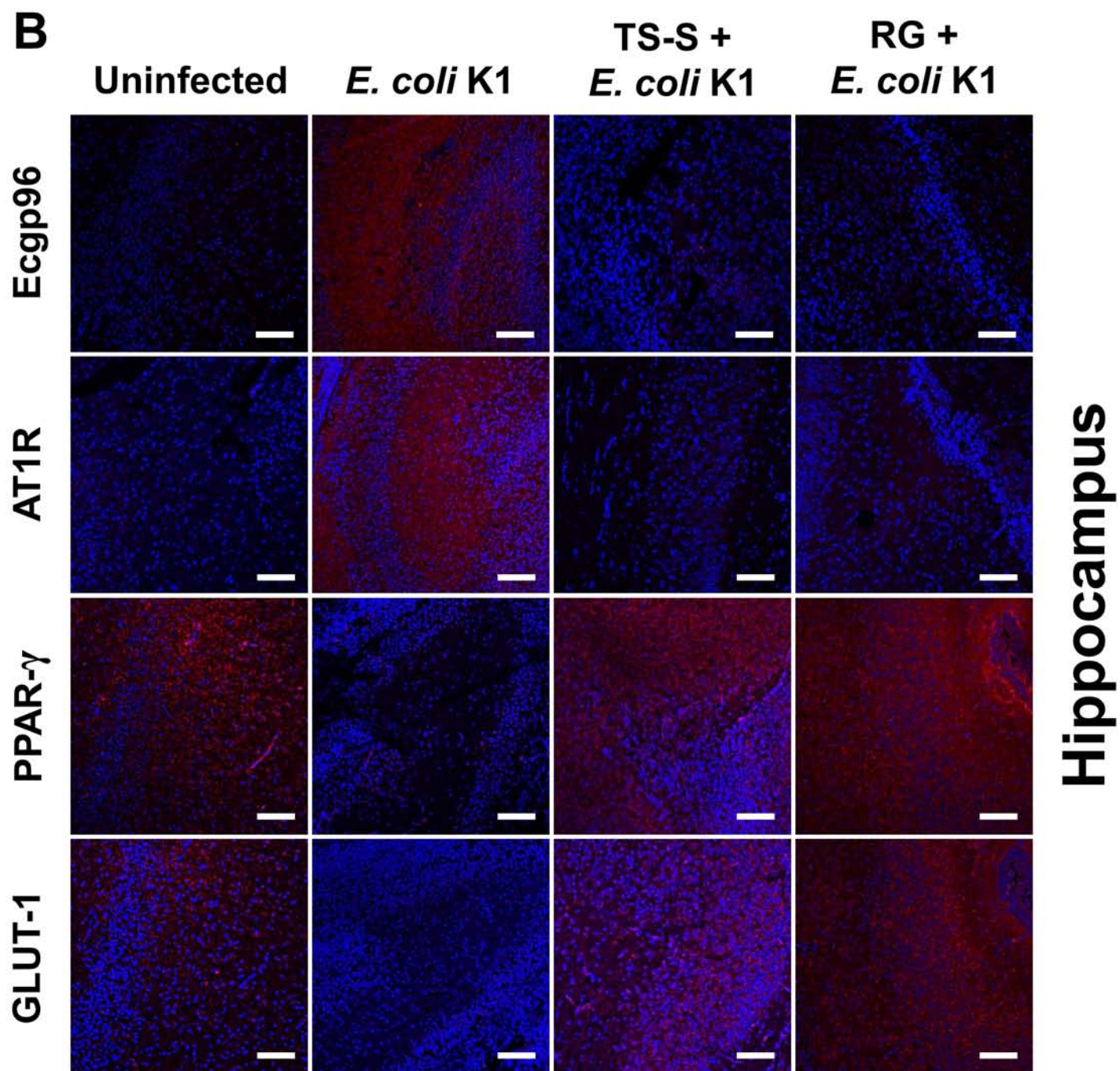


Fig. 6

C

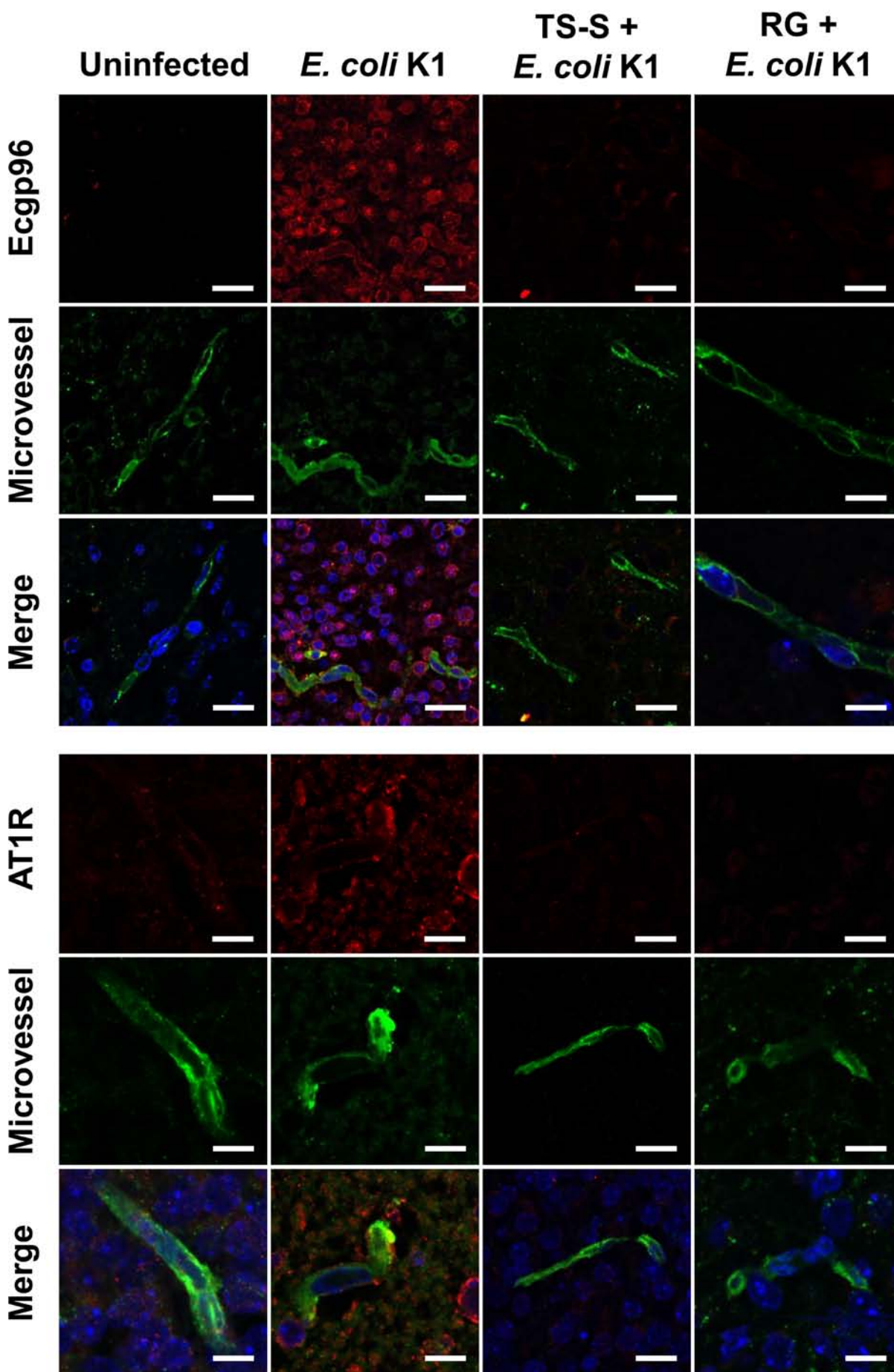


Fig. 6

D

